Inventor(s): Naesby

Attorney Docket No.: 108382-08067

I. AMENDMENTS TO THE CLAIMS

Claims 1 to 32. (Canceled)

Claim 33. (Currently Amended) The method according to claim 37 A method for determining the presence or amount of a nucleic acid A, comprising: (a) forming a triple stranded complex between said nucleic acid A, a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and a binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C, wherein said one or more nucleic acid A binding probes C, in the aggregate, comprise a base sequence different from the base sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of probe B; and (b) determining the presence or amount of said triple stranded complex as an indication of the presence or amount of nucleic acid A, wherein said nucleic acid A binding probe B comprises a binding region of 4 to 10 bases.

Claims 34 to 36. (Canceled)

Claim 37. (Currently Amended) The method according to claim 33, A method for determining the presence or amount of a nucleic acid A, comprising: (a) forming a triple stranded complex between said nucleic acid A, a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and a binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C, wherein said one or more nucleic acid A binding probes C, in the aggregate, comprise a base sequence different from the base sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of probe B; and (b) determining the presence or amount of said triple stranded complex as an indication of the presence or amount of nucleic acid A, and wherein said triple stranded complex comprises two different nucleic acid A binding probes C which bind to different regions of nucleic acid A in the triple stranded region.

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Claim 38. (Previously Presented) The method according to claim 37 wherein said two different nucleic acid A binding probes C of the triple stranded complex form an aggregate binding region which is comprised of two distinct triple helical binding regions, wherein each distinct triple helical binding region is formed from the binding of the two different probes C each to a distinct, non-overlapping, region on nucleic acid A.

Claim 39. (Previously Presented) The method according to claim 38 wherein said two different nucleic acid A binding probes C bind juxtaposed on nucleic acid A.

Claim 40. (Previously Presented) The method according to claim 38 wherein said triple stranded complex is at least six bases in length and each of the two different nucleic acid A binding probes C individually contribute at least one but less than eleven bases to said triple stranded complex.

Claim 41. (Currently Amended) The method according to claim 33 claim 37, wherein said binding region of nucleic acid A binding probe B comprises only pyrimidine bases but the aggregate binding region of the one or more nucleic acid A binding probes C comprises at least one non-pyrimidine base.

Claims 42 to 43. (Canceled)

Claim 44. (Currently Amended) The method according to claim 33 claim 37, wherein at least one of said nucleic acid A binding probes has been chemically modified to destabilize triple helix formation occurring by either of: (a) two nucleic acid A binding probes B binding to one nucleic acid A; or (b) two of said nucleic acid A binding probes C binding with one nucleic acid A.

Claim 45. (Currently Amended) The method according to claim 33 A method for determining the presence or amount of a nucleic acid A, comprising: (a) forming a triple stranded complex between said nucleic acid A, a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and a binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C, wherein said one or more nucleic acid A binding probes C, in the aggregate, comprise a base

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sequence different from the base sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of probe B; and (b) determining the presence or amount of said triple stranded complex as an indication of the presence or amount of nucleic acid A, and wherein a first nucleic acid not to be determined is differentiated from said nucleic acid A by a difference in the base sequence located outside the binding region of the nucleic acid A binding probe B but within the aggregate binding region of the one or more nucleic acid A binding probes C.

Claim 46. (Currently Amended) The method according to claim 33 claim 37, wherein a first nucleic acid not to be determined is differentiated from said nucleic acid A by a difference in the base sequence located within the binding region of the nucleic acid A binding probe B.

Claim 47. (Currently Amended) The method according to claim 33 A method for determining the presence or amount of a nucleic acid A, comprising: (a) forming a triple stranded complex between said nucleic acid A, a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and a binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C, wherein said one or more nucleic acid A binding probes C, in the aggregate, comprise a base sequence different from the base sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of probe B; and (b) determining the presence or amount of said triple stranded complex as an indication of the presence or amount of nucleic acid A, and wherein a reaction mixture is used for forming the triple stranded complex, said reaction mixture containing a competitive probe D which can compete with at least one nucleic acid A binding probe C in binding to nucleic acid A, but which is incapable of participating in the formation of the triple stranded complex.

Claim 48. (Currently Amended) The method according to claim 33 claim 37, wherein at least one of said nucleic acid A binding probes is a nucleic acid analogue.

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Claim 49. (Previously Presented) The method according to claim 48 wherein said nucleic acid analogue is a peptide nucleic acid.

Claim 50. (Currently Amended) The method according to claim 33 claim 37, wherein at least one of said nucleic acid A binding probes is a polymer of the general Formula I

$$Q = \begin{bmatrix} C_1 \\ C_1 \end{bmatrix}_{x1}^{B_1} D_1 = \begin{bmatrix} C_1 \\ C_2 \end{bmatrix}_{D^x}^{C_1} D_1 = \begin{bmatrix} C_1 \\ C_2 \end{bmatrix}_{D^x}^{C_1}$$

Formula I

wherein

n is an integer of from at least 3,

x is an integer of from 2 to n-1,

each of L¹-Lⁿ is a ligand independently selected from the group consisting of hydrogen, hydroxy, (C₁-C₄)alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties, wherein at least one of L¹-Lⁿ, preferably at least one of L²-Lⁿ-¹ is a non-nucleobase electron acceptor or a donor moiety and at least 2 of L¹-Lⁿ being a nucleobase binding group, or a naturally or non-naturally occurring nucleobase;

each of C¹-C¹ is (CR⁶R²)_y (preferably CR⁶R², CHR⁶CHR² or CR⁶R²CH₂) where R⁶ is hydrogen and R² is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R⁶ and R² are independently selected from the group consisting of hydrogen, (C₁-C₆)alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C₁-C₆)alkoxy, (C₁-C₆)alkylthio, NR³R⁴ and SR⁵, where R³ and R⁴ are as defined below, and R⁵ is hydrogen, (C₁-C₆)alkyl, hydroxy, (C₁-C₆)alkoxy, or (C₁-C₆)alkylthio-substituted (C₁-C₆)alkyl or R⁶ and R² taken together complete an alicyclic or heterocyclic system; or C¹-C¹ is CO, CS, CNR³;

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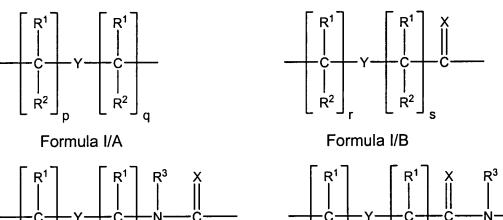
each of D¹-D¹ is (CR⁶R²)_z (preferably CR⁶R², CHR⁶CHR² or CH₂CR⁶R²) where R⁶ and R² are as defined above:

each of y and z is zero or an integer from 1 to 10, the sum y + z being at least 2, preferably greater than 2, but not more than 10;

each of G¹-Gⁿ⁻¹ is -NR³CO-, -NR³CS-, -NR³SO- or -NR³SO²-, in either orientation, where R³ is as defined below;

each of A¹-Aⁿ and B¹-Bⁿ are selected such that:

- (a) A^1 - A^n is a group of formula (I/A), (I/B), (I/C) or (I/D), and B^1 - B^n is N or R^3 N+; or
- (b) A¹-Aⁿ is a group of formula (I/D) and B¹-Bⁿ is CH;



 $\begin{bmatrix} 1 \\ R^2 \end{bmatrix}_r \begin{bmatrix} 1 \\ R^2 \end{bmatrix}_s$

Formula I/C

 $\begin{array}{c|c}
R^1 \\
C \\
C \\
R^2
\end{array}$ $\begin{array}{c|c}
R^1 \\
C \\
C \\
R^2
\end{array}$ $\begin{array}{c|c}
R^3 \\
C \\
R^2
\end{array}$

Formula I/D

wherein:

X is O, S, Se, NR^3 , CH_2 or $C(CH_3)_2$;

Y is a single bond, O, S or NR⁴;

each of p and q is zero or an integer from 1 to 5, (the sum p+q being preferably not more than 5);

each of r and s is zero or an integer from 1 to 5, (the sum r+s being preferably not more than 5);

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each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4) alkyl which may be hydroxy- or (C_1-C_4) alkoxy- or (C_1-C_4) alkylthio-substituted, hydroxy, (C_1-C_4) alkoxy, (C_1-C_4) alkylthio, amino and halogen; and

each R^3 and R^4 is independently selected from the group consisting of hydrogen, (C_1-C_4) alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4) alkyl, hydroxy, (C_1-C_6) -alkylthio and amino;

Q and I is independently selected from -CO₂H, -CONR'R", -SO₃H or -SO₂NR'R" or an activated derivative of -CO₂H or -SO₃H and -NR'R"

where R', R" and R" are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers and as well as nucleic acid binding moieties and each of x1 and y1 is an integer of from 0 to 10.

Claim 51. (Currently Amended) A method according to claim 33 claim 37, wherein at least one of said nucleic acid A binding probes is a polymer of the general Formula I

$$Q = \begin{bmatrix} C_1 \\ C_1 \end{bmatrix}_{x1}^{B_1} D_1 = \begin{bmatrix} C_1 \\ C_2 \end{bmatrix}_{B^x} D_x = \begin{bmatrix} C_1 \\ C_2 \end{bmatrix}_{D^x}^{G^x} D_x = \begin{bmatrix} C_1 \\ C_2 \end{bmatrix}_{D^x}^{G^x} D_x = \begin{bmatrix} C_1 \\ C_2 \end{bmatrix}_{y1}^{G^x}$$

Formula I

wherein

n is an integer of from at least 3,

x is an integer of from 2 to n-1,

each of L¹-Lⁿ is a ligand independently selected from the group consisting of hydrogen, hydroxy, (C₁-C₄)alkanoyl, naturally occurring nucleobases, non-naturally

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occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties, wherein at least one of L¹-Lⁿ, preferably at least one of L²-Lⁿ⁻¹ is a non-nucleobase electron acceptor or a donor moiety and at least 2 of L¹-Lⁿ being a nucleobase binding group, or a naturally or non-naturally occurring nucleobase;

each of C^1 - C^n is $(CR^6R^7)_y$ (preferably CR^6R^7 , CHR^6CHR^7 or $CR^6R^7CH_2$) where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, $(C_1$ - C_6)alkyl, aryl, aralkyl, heteroaryl, hydroxy, $(C_1$ - C_6)alkoxy, $(C_1$ - C_6)alkylthio, NR^3R^4 and SR^5 , where R^3 and R^4 are as defined below, and R^5 is hydrogen, $(C_1$ - C_6)alkyl, hydroxy, $(C_1$ - C_6)alkoxy, or $(C_1$ - C_6)alkyl or R^6 and R^7 taken together complete an alicyclic or heterocyclic system; or C^1 - C^n is CO. CS, CNR^3 ;

each of D¹-D¹ is (CR⁶R⁷)_z (preferably CR⁶R⁷, CHR⁶CHR⁷ or CH₂CR⁶R⁷) where R⁶ and R⁷ are as defined above:

each of y and z is zero or an integer from 1 to 10, the sum y + z being at least 2, preferably greater than 2, but not more than 10;

each of G^1 - G^{n-1} is -NR³CO-, -NR³CS-, -NR³SO- or -NR³SO²⁻, in either orientation, where R³ is as defined below;

each of A¹-Aⁿ and B¹-Bⁿ are selected from (Ia), (Ib) or (Ic) such that:

- (Ia): B^1 - B^n is N and A^1 - A^n is -CO-(CH₂)₆-
- (lb): B^1 - B^n is N and A^1 - A^n is -CO-NR³-(CH₂)₂-
- (Ic): B^1 - B^n is CH and A^1 - A^n is -NR³-CO-(CH₂)₂-

Q and I is independently selected from -CO₂H, -CONR'R", -SO₃H or -SO₂NR'R" or an activated derivative of -CO₂H or -SO₃H and -NR'R"

where R', R" and R" are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both

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oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers and as well as nucleic acid binding moieties and each of x1 and y1 is an integer of from 0 to 10.

Claim 52. (Currently Amended) The method according to claim 33 claim 37, wherein at least one of said nucleic acid A binding probes comprise at least one monomer subunit of general Formula III

Formula III

each of L is a ligand independently selected from the group consisting of hydrogen, hydroxy, (C₁-C₄)alkanoyl, naturally occurring nucleobases, non-naturally occurring nucleobases, aromatic moieties, DNA intercalators, nucleobase-binding groups, heterocyclic moieties, reporter ligands and chelating moieties, wherein at least one of L is a non-nucleobase electron acceptor or a donor moiety and at least 2 of L being a nucleobase binding group, or a naturally or non-naturally occurring nucleobase;

each of C is $(CR^6R^7)_y$ where R^6 is hydrogen and R^7 is selected from the group consisting of the side chains of naturally occurring alpha amino acids, or R^6 and R^7 are independently selected from the group consisting of hydrogen, (C_1-C_6) alkyl, aryl, aralkyl, heteroaryl, hydroxy, (C_1-C_6) alkoxy, (C_1-C_6) alkylthio, NR^3R^4 and SR^5 , where R^3 and R^4 are as defined below, and R^5 is hydrogen, (C_1-C_6) alkyl, hydroxy, (C_1-C_6) alkoxy, or (C_1-C_6) alkylthio-substituted (C_1-C_6) alkyl or R^6 and R^7 taken together complete an alicyclic or heterocyclic system; or C is CO, CS, CNR^3 ;

each of D is $(CR^6R^7)_z$ where R^6 and R^7 are as defined above; each of y and z is zero or an integer from 1 to 10, the sum y + z being at least 2;

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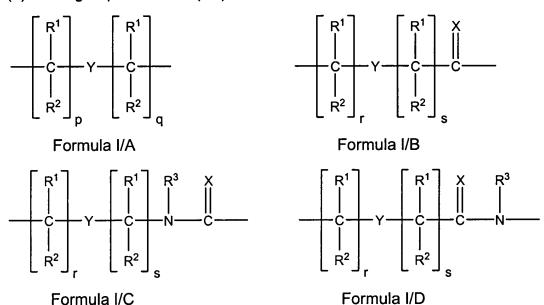
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each of G is -NR³CO-, -NR³CS-, -NR³SO- or -NR³SO²⁻, in either orientation, where R³ is as defined below;

each of A and B are selected such that:

- (a) A is a group of formula (I/A), (I/B), (I/C) or (I/D), and B is N or \mathbb{R}^3N+ ; or
- (b) A is a group of formula (I/D) and B is CH;



wherein:

X is O, S, Se, NR^3 , CH_2 or $C(CH_3)_2$; Y is a single bond, O, S or NR^4 ; each of p and q is zero or an integer from 1 to 5, each of r and s is zero or an integer from 1 to 5,

each R^1 and R^2 is independently selected from the group consisting of hydrogen, (C_1-C_4) alkyl which may be hydroxy- or (C_1-C_4) alkoxy- or (C_1-C_4) alkylthio-substituted, hydroxy, (C_1-C_4) alkoxy, (C_1-C_4) alkylthio, amino and halogen; and

each R^3 and R^4 is independently selected from the group consisting of hydrogen, (C_1-C_4) alkyl, hydroxy- or alkoxy- or alkylthio-substituted (C_1-C_4) alkyl, hydroxy, (C_1-C_6) -alkylthio and amino;

where R', R" and R" are independently selected from the group consisting of hydrogen, alkyl, amino protecting groups, reporter ligands, intercalators, chelators, peptides, proteins, carbohydrates, lipids, steroids, nucleosides, nucleotides, nucleotide diphosphates, nucleotide triphosphates, oligonucleotides, including both

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oligoribonucleotides and oligodeoxyribonucleotides, oligonucleosides and soluble and non-soluble polymers and as well as nucleic acid binding moieties and each of x1 and y1 is an integer of from 0 to 10.

Claim 53. (Currently Amended) The method according to claim 33 claim 37, wherein said triple stranded complex is more thermostable than a triple stranded complex formed by two nucleic acid A binding probes B binding with one nucleic acid A.

Claim 54. (Currently Amended) The method according to claim 33 claim 37, wherein said triple stranded complex is more thermostable than a triple stranded complex formed from either of: (a) two nucleic acid A binding probes B binding with one nucleic acid A; or (b) two of said nucleic acid A binding probes C binding with one nucleic acid A.

Claims 55 to 56. (Canceled)

Claim 57. (Currently Amended) The complex according to claim 59 A triple stranded complex comprising a nucleic acid A, a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and a binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C wherein said one or more nucleic acid A binding probes C, in the aggregate, comprise a base sequence different from the sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of nucleic acid A binding probe B, wherein said nucleic acid A binding probe B comprises a binding region of 4 to 10 bases.

Claim 58. (Canceled)

Claim 59. (Currently Amended) The complex according to claim 57 A triple stranded complex comprising a nucleic acid A, a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and a binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C wherein said one or more nucleic acid A binding probes C, in the aggregate, comprise a base sequence different

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from the sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of nucleic acid A binding probe B, and wherein said triple standard complex comprises two different nucleic acid A binding C probes.

Claim 60. (Previously Presented) The complex according to claim 59 wherein said two different nucleic acid A binding probes C of the triple stranded complex form an aggregate binding region which is comprised of two distinct triple helical binding regions, wherein each distinct triple helical binding region is formed from the binding of the two different nucleic acid A binding probes C each to a distinct, non-overlapping, region on nucleic acid A.

Claim 61. (Previously Presented) The complex according to claim 60 wherein said two different nucleic acid A binding probes C bind juxtaposed on nucleic acid A.

Claim 62. (Currently Amended) The complex according to claim 57 claim 59, wherein said binding region of nucleic acid A binding probe B comprises only pyrimidine bases but the aggregate binding region of the one or more nucleic acid A binding probes C comprises at least one non-pyrimidine base.

Claim 63 to 64. (Canceled)

Claim 65. (Currently Amended) The complex according to claim 57 claim 59, wherein at least one of said nucleic acid A binding probes is a nucleic acid analogue.

Claim 66. (Currently Amended) The complex according to claim 57 claim 59, wherein said nucleic acid analogue is a peptide nucleic acid.

Claim 67. (Currently Amended) The complex according to claim 57 claim 59, wherein said triple stranded complex is more thermostable than a triple stranded complex formed by two nucleic acid A binding probes B binding with one nucleic acid A.

Claim 68. (Currently Amended) The complex according to claim 57 claim 59, wherein said triple stranded complex is more thermostable than a triple stranded complex

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formed from either of: (a) two nucleic acid A binding probes B binding with one nucleic acid A; or (b) two of said nucleic acid A binding probes C binding with one nucleic acid A.

Claims 69 to 70. (Canceled)

Claim 71. (Currently Amended) The method according to claim 73 A method of forming a triple stranded binding complex comprising reacting a nucleic acid molecule A with a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C, wherein said one or more nucleic acid A binding probes C, in the aggregate, comprise a base sequence different from the sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of nucleic acid A binding probe B, wherein said nucleic acid A binding probe B comprises a binding region of 4 to 10 bases.

Claim 72. (Canceled)

Claim 73. (Currently Amended) The method according to claim 71 A method of forming a triple stranded binding complex comprising reacting a nucleic acid molecule A with a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C, wherein said one or more nucleic acid A binding probes C, in the aggregate, comprise a base sequence different from the sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of nucleic acid A binding probe B, and wherein said triple stranded complex comprises two different nucleic acid A binding probes C.

Claim 74. (Currently Amended) The method according to claim 71 A method of forming a triple stranded binding complex comprising reacting a nucleic acid molecule A with a

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nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and binding region which binds to nucleic acid A, and one or more nucleic acid A binding probes C, wherein said one or more nucleic acid A binding probes C, in the aggregate, comprise a base sequence different from the sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of nucleic acid A binding probe B, wherein two different nucleic acid A binding probes C of the triple stranded complex form an aggregate binding region which is comprised of two distinct triple helical binding regions, and wherein each distinct triple helical binding region is formed from the binding of the two different nucleic acid A binding probes C each to a distinct, non-overlapping, region on nucleic acid A.

Claim 75. (Previously Presented) The method according to claim 74 wherein said two different nucleic acid A binding probes C bind juxtaposed on nucleic acid A.

Claim 76. (Currently Amended) The method according to claim 71 claim 73, wherein said binding region of nucleic acid A binding probe B comprises only pyrimidine bases but the aggregate binding region of the one or more nucleic acid A binding probes C comprises at least one non-pyrimidine base.

Claim 77. (Currently Amended) The method according to claim 71 claim 73, wherein said nucleic acid A binding probe B is bound to nucleic acid A via Hoogsteen base pairing and the one or more nucleic acid A binding probes C are bound to nucleic acid A via Watson Crick base pairing.

Claim 78. (Currently Amended) The method according to claim 71 claim 73, wherein at least one of said nucleic acid A binding probes is a nucleic acid analogue.

Claim 79. (Currently Amended) The method according to claim 71 claim 73, wherein said nucleic acid analogue is a peptide nucleic acid.

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Claim 80. (Currently Amended) The method according to elaim 71 claim 73, wherein said triple stranded complex is more thermostable than a triple stranded complex formed by two nucleic acid A binding probes B binding with one nucleic acid A.

Claim 81. (Currently Amended) The method according to claim 71 claim 73, wherein said triple stranded complex is more thermostable than a triple stranded complex formed from either of: (a) two nucleic acid A binding probes B binding with one nucleic acid A; or (b) two of said nucleic acid A binding probes C binding with one nucleic acid A.

Claim 82. (Previously Presented) A method for determining the presence or amount of a nucleic acid A, comprising: (a) forming a triple stranded complex between said nucleic acid A, a nucleic acid A binding probe B, said nucleic acid A binding probe B having a base sequence and a binding region which binds to nucleic acid A, and two nucleic acid A binding probes C, wherein said two nucleic acid A binding probes C, in the aggregate, comprise a base sequence different from the base sequence of nucleic acid A binding probe B and an aggregate binding region wherein said aggregate binding region, which binds to nucleic acid A, is longer as compared with the binding region of nucleic acid A binding probe B; and (b) determining the presence or amount of said nucleic acid A by measuring for the presence or amount of said triple stranded complex.

Claim 83. (Previously Presented) The method according to claim 82 wherein said two nucleic acid A binding probes C of the triple stranded complex are different and form an aggregate binding region which is comprised of two distinct triple helical binding regions, wherein each distinct triple helical binding region is formed from the binding of the two different nucleic acid A binding probes C each to a distinct, non-overlapping, region on nucleic acid A.

Claim 84. (Previously Presented) The method according to claim 83 wherein said two different nucleic acid A binding probes C bind juxtaposed on nucleic acid A.

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Claim 85. (Previously Presented) The method according to claim 83 wherein said triple stranded complex is at least six bases in length and each of the two different nucleic acid A binding probes C individually contribute at least one but less than eleven bases to said triple stranded complex.

Claim 86. (New) The method according to claim 37, wherein said binding region of nucleic acid A binding probe B has an asymmetric base sequence.

Claim 87. (New) The method according to claim 37, wherein said binding region of nucleic acid A binding probe B has a symmetric base sequence.

Claim 88. (New) The method according to claim 37, wherein said aggregate binding region of the one or more nucleic acid A binding probes C has a length of at least 6 bases.

Claim 89. (New) The method according to claim 37, wherein said nucleic acid A binding probe B is bound to nucleic acid A via Hoogsteen base pairing and the one or more nucleic acid A binding probes C are bound to nucleic acid A via Watson Crick base pairing.

Claim 90. (New) The method according to claim 37, wherein at least one of said nucleic acid A binding probes is labeled and the presence of said label in the triple stranded complex is used for determining the presence or amount of the nucleic acid A.

Claim 91. (New) The complex according to claim 59, wherein said aggregate binding region of the one or more nucleic acid A binding probes C has a length of at least 6 bases.

Claim 92. (New) The complex according to claim 59, wherein said nucleic acid A binding probe B is bound to nucleic acid A via Hoogsteen base pairing and the one or more nucleic acid A binding probes C are bound to nucleic acid A via Watson Crick base pairing.

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Claim 93. (New) The complex according to claim 59, wherein at least one of said nucleic acid A binding probes is labelled and the presence of said label in the triple stranded complex is used for determining the presence or amount of the nucleic acid A.

Claim 94. (New) The method according to claim 73, wherein said aggregate binding region of the one or more nucleic acid A binding probes C has a length of at least 6 bases.

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